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Prevalence and co-infection of *Toxoplasma gondii* and *Human Immunodeficiency Virus* (HIV) infection among women of child-bearing age in Osun state Nigeria

Abstract

This study assessed the epidemiology of *Toxoplasma gondii* and *Human immunodeficiency Virus* and their co-infection infection in women of child-bearing age in communities in Osun State, Nigeria. The study also determines the factors that facilitate the organisms among the studied groups and possible interaction between the prevalence of infection and the risk factors. These were done with a view to providing baseline information on the mode of *T. gondii* and HIV transmission. Sera were analyzed for the presence of IgG and IgM antibodies against *T. gondii* by commercially available enzyme linked immunosorbent assay (ELISA) kit (Demeditec Diagnostics GmbH, Germany) conducted according to the manufacturer's instructions. The optical densities of wells were measured by a photometer at a wavelength of 450 nm and the detection of HIV was done using (Determine) rapid immunochromatographic (IC) test which are in strip form. Determination of the blood groups of the donors was done using commercially prepared Anti sera A, B, AB and anti D that determine the Rhesus blood group.

The overall prevalence of *T. gondii* among the women of child-bearing age in selected six communities in Osun state was 74.2%, while the overall prevalence of HIV among the women was 2.6% and co-infection of *T. gondii* and HIV was 1.5%. The prevalence of *T. gondii* was lowest (57.8%) among women from Ile Ife, a peri-urban community and highest (100%) in women residing in Alajue, a rural community. The prevalence of *T. gondii* infection was significantly higher (p= 0.001**)** among Islamic women (85.9%) than in Christian women (68.2%) while the prevalence of HIV (2.7%) in Christianity and co-infection of *T. gondii* and HIV (2.3%) was higher among Islam. The highest prevalence of *T. gondii* (83.6%) was recorded in women with primary education while the lowest of (58.7%) was recorded in women with tertiary education (p =0.037**)** while the highest prevalence of HIV infection of 2.9% and co-infection of 1.8% was recorded in secondary school level.

The highest prevalence of *T. gondii* (78.5%) was recorded in women that reside in rural area and the lowest (67.5%) was recorded in women that reside in peri-urban area **(**p=0.016) and also the prevalence of HIV (4.5%) **(**p= 0.045**)** and co-infection of *T. gondii* and HIV (3.2%) **(**p=0.025) was higher in peri-urban area. The highest prevalence of 84.0% of *T. gondii* was recorded in house wives while the lowest value of 59.3% was recorded in civil servant and the highest prevalence of HIV infection of 7.7% in house wives and co-infection of 3.3% was recorded in trading.

The highest prevalence of *T. gondii* infection of 100.0% was recorded in women with blood group AB negative while the lowest prevalence of 30.0% was recorded in women with blood group A negative and the highest prevalence of HIV infection of 6.3% was recorded in women with blood group B negative and O negative each. Co-infection has highest prevalence of 6.3% in blood group B negative.

The highest prevalence of 77.1% of *T. gondii* was recorded in women with one miscarriage while the lowest value of 73.3% was recorded in women with two miscarriages and the highest prevalence of HIV infection of 5.7% was recorded in women with one miscarriage while the lowest prevalence of 2.5% was recorded in women with no miscarriage. The highest prevalence of 100.0% of *T. gondii* and 2.7% of HIV infection was recorded in women with no pregnancy and the co-infection 1.6% was recorded in women no pregnancy.

The study concluded that there was high prevalence of *T. gondii* infection (74.2%) and also implicates HIV (2.6%) and co-infection of both (1.5%) in the studied population. Hence, there is the need for health education and create awareness of the diseases and its transmission to women of reproductive age group in general and pregnant women in particular to reduce the risk of *T. gondii and* HIV in pregnant women.

Keywords: pregnant women, HIV, *T. gondii,* toxoplasmosis

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Abbreviations: HIV,human immunodeficiency virus; NGO;non-governmental organization

Introduction

Toxoplasmosis is caused by the obligate intracellular protozoan *Toxoplasma gondii.* It is one of the most prevalent chronic infections affecting one third of the world's human population. In many developing countries as well as our country, the exact prevalence of toxoplasmosis among pregnant women is not well recognized. *T. gondii* infection can cause a more serious progression when accompanied with some other infection.[1–3] People with HIV infection has a very high burden of *T gondii* infection, especially in sub-Saharan Africa, and emphasizes the importance of routine surveillance for *T. gondii* infection in all HIV-infected people.[4,5] In HIV-positive individuals, *T. gondii* causes severe opportunistic infections, which is of major public health concern as it results in physical and psychological disabilities. *Toxoplasma gondii* infection in AIDS patients and pregnant women causes severe encephalitis, and neurologic diseases, and can affect

the heart, liver, inner ears, and eyes (chorioretinitis), it also causes cervical lymphadenopathy or ocular disease in HIV infected immune competent individuals.[6] Therefore, this study was conducted to determine the prevalence of *T. gondii and* HIV *virus* among women of childbearing age in Osun State, Nigeria.

Materials and methods

Ethical consideration

The research protocol was submitted and approved by the Ethics and Research committee of the Institute of Public Health, College of Health sciences, Obafemi Awolowo University, Ile Ife Osun State. Nigeria, before the commencement of the study. Written informed consents were obtained from all women and confidentiality was assured by using codes.

Study area

The study was conducted in six randomly selected communities (Ile Ife, Ifewara, Edunagbon, Ikire, Alajue and Erin Ijesa) within Osun State. The State is situated in the tropical rain forest zone. It covers an area of approximately 14,875 sq km and lies between latitude 7° 30′ 0″ N and longitude 4° 30′ 0″ E. Majority of the inhabitants are skilled workers, eg. civil servants and artisans while others are unskilled workers, e.g. peasant farmers, traders and transport workers. People of Osun State practice Islam, Christianity and their ancient religion, the traditional faith.

Study population

The study populations comprise women of child-bearing age (pregnant and non-pregnant) who attend monthly clinic organized by a non-governmental organization (NGO). The Women who brought their children for medical attention were approached and the purpose of the study was discussed with them. Thereafter, the procedure to be taken was explained to them. The women were informed that only those that signed the consent form will be allowed to participate in the study. They were also informed that participation is voluntary and that they can withdraw from participating at any point if they wish.

Study design

The study was conducted between March 2019 and September 2019. A total of 391 sera were collected and prior to the collection of the blood samples structured questionnaire designed to collect basic demographic information including age, residential location, occupation and educational level and other information as regards the study.

Sample size

A total of 391 samples were collected after ethical clearance and verbal consent were obtained from the subjects.

Sample collection and processing

Five (5) ml of blood was collected by venipuncture into blood collection tube and serum of the samples were separated by centrifuging at 3000 rpm for 5 mins and stored at -20^{0}C for serological analysis to detect the presence of IgG and IgM.

Laboratory analysis (ELISA Technique)

Sera were analyzed for the presence of IgG and IgM antibodies against *T. gondii* by commercially available enzyme linked immunosorbent assay (ELISA) kit (Demeditec Diagnostics GmbH, Germany) conducted according to the manufacturer's instructions. The kit has reported sensitivity and specificity of 98% and 99%, respectively. The optical densities of wells were measured by a photometer at a wavelength of 450 nm.

Screening for Human immunodeficiency Virus (HIV)

Test to screen the 391 blood samples for HIV was done using (Determine) rapid immunochromatographic (IC) test which are in strip form. The SMI One Step HIV Test Device (Serum/Plasma) is a qualitative, lateral flow immunoassay for the detection of HIV antibody in serum or plasma.

Determination of Blood group of the donors

Determination of the blood groups of the donors was done using Anti sera A, B, AB and anti D that determine the Rhesus factor as follows:

A glass slide was marked as follows Anti-A, Anti-B, Anti D *and* each division was pipette into as follows: Anti-A*:* 1 volume anti-A serum, 1 volume donor's capillary blood, *Anti-B:* 1 volume anti-B serum, 1 volume donor's capillary blood, Anti-AB*:* 1 volume anti-AB serum, 1 volume donor's capillary blood, Anti-D*:* 1 volume anti-D serum, 1 volume donor's capillary blood. The contents of each division were mixed using a clean piece of stick for each. The slide was tilted from side to side, looking for agglutination and the results recorded after 2 minutes

Data analysis

Analysis of the data was done using appropriate Statistical Package for the Social Sciences tool version 21.0. The prevalence was calculated as the number of serologically positive samples divided by the total number of samples tested. The Chi-square test was used to determine associations between positivity and socio-demographic factors. The strength of the associations was assessed by odds ratios and 95% confidence intervals (CI) were calculated.

Results

The overall prevalence of *T. gondii* among the women of child-bearing age in selected six communities in Osun state was 74.2%, while the overall prevalence of HIV among the women was 2.6% and co-infection of *T. gondii* and HIV was 1.5%. A total of 391 women of child-bearing age participated in the study. Majority of the women that participated, 28.6% (112/391) were in the age range 25-29 years, 50.1% (196/391) were artisans, 69.6% (272/391) attended secondary education and 60.6% (237/391) reside in rural area.

The highest prevalence of *T. gondii* infection of 94.4% was recorded in women age range≥40 years while the lowest prevalence of 63.2% was recorded in women age range 15-19 years and the highest prevalence of HIV infection of 4.8% in 20-24years and co-infection of 4.3% was recorded in women age range 35-39 years and the lowest prevalence of HIV of 2.7% and co-infection of 1.8% was recorded in women age group 25-29years (Table 1).

In rural area, the highest prevalence of (78.5%) of *T. gondii* infection of was recorded while the lowest of 67.5% was recorded in women that reside in peri-urban area. There was significant difference in prevalence of infection across the residential place of the women (p = 0.016), while, HIV has the highest prevalence of 4.5% (p= 0.045) and co-infection 3.2% (p=0.025) among the women that reside in peri-urban area.

Traditional has the highest prevalence of 100.0% of *T. gondii* followed by 85.9% in Islam while the lowest value of 68.2% was

recorded in Christianity. There is significance difference in the prevalence of *T. gondii* in the religions (P= 0.001), and the highest prevalence of HIV infection of 2.7% was recorded in Christianity whiles 2.3% co-infection was recorded in Islam.

Table 1 Prevalence of Toxo*plasma gondii*, *Human Immunodeficiency Virus* and co-infections according to socio-demographic factors among women of child–bearing age in selected communities in Osun State (N=391)

Variables	No examined	No infected (%)HIVs	No infected (%) (*T. gondii*)	No infected (%) HIV and TOXO	P value
Age (years)					HIV (X^2)=6,097, p=0.297
15-19	19	0 (0.0)	12 (63.2)	0(0.0)	HIV TOXO (X^2)=4.571, p=0.470,
20-24	104	5 (4.8)	81 (77.9)	2(1.9)	TOXO= (X^2)=7.128, p=0.211
25-29	112	3 (2.7)	79 (70.5)	2(1.8)	
30-34	92	0 (0.0)	69 (75.0)	0(0.0)	
35-39	46	2 (4.3)	32 (69.6)	2(4.3)	
≥40	18	0 (0.0)	17 (94.4)	0 (0.0)	
Residence					HIV (X^2)= 4,029, p=0.045
Peri-urban	154	7 (4.5)	104 (67.5)	5 (3.2)	HIVTOXO (X^2)= 4.930, p=0.025,
Rural	237	3 (1.3)	186 (78.5)	1 (0.4)	TOXO (X^2)= 5.840, P=0.016
Religion					HIV (X^2)=0.092, p=0.955
Christianity	261	7 (2.7)	178 (68.2)	3(1.1)	HIVTOXO(X^2)= 0.842, p=0.656
Islam	128	3 (2.3)	110 (85.9)	3 (2.3)	TOXO (X^2)=14.805, p=0.001
Traditional	2	0 (0.0)	2 (100.0)	0 (0.0)	
Occupation					HIV (X^2)= 2.787, p=0.594
Trading	150	5 (3.3)	118 (78.7)	5 (3.3)	HIVTOXO(X^2)= 25.275, p=0.260
Artisan	196	4 (2.0)	141 (71.9)	1 (0.5)	TOXO (X^2)= 6.055 P=0,195
Student	5	0 (0.0)	4(80.0)	0 (0.0)	
Civil Servant	27	0 (0.0)	16 (59.3)	0 (0.0)	
House Wives	13	1 (7.7)	11 (84.0)	0 (0.0)	
Education					HIV (X^2)= 0.781, p=0.854
None	18	0 (0.0)	14 (77.8)	0 (0.0)	HIVTOXO(X^2)= 1.193, p=0.755
Primary	55	1 (1.8)	46 (83.6)	1 (1.8)	TOXO (X^2)= 8.475 P=0.037
Secondary	272	8(2.9)	203(74.6)	5 (1.8)	
Tertiary	46	1 (2.2)	27 (58.7)	0 (0.0)	
Community					HIV (X^2)= 13.993, p=0.016
ALAJUE	27	0 (0.0)	27 (100.0)	0 (0.0)	HIVTOXO(X^2)= 7.336, p=0.197
EDUNABON	50	0 (0.0)	31 (62.0)	0 (0.0)	TOXO (X^2)= 31.918 P=0,000
ERIN- IJESHA	64	0 (0.0)	49 (76.6)	0 (0.0)	
ILE IFE	90	7 (7.8)	52(57.8)	4 (4.4)	
IFEWARA	86	2 (2.3)	73 (84.9)	1 (1.2)	
IKIRE	74	1 (1.4)	58(78.4)	1 (1.4)	
Total	391	10 (2.6)	290 (74.2)	6 (1.5)	

TOXO, Toxo*plasma gondii;* HIV, *Human Immunodeficiency Virus;* HIV TOXO, Toxo*plasma gondii and Human Immunodeficiency Virus*

The highest prevalence of 84.0% of *T. gondii* was recorded in house wives followed by 80.0% in student while the lowest value of 59.3% was recorded in civil servant while the highest prevalence of HIV infection of 7.7% in house wives and co-infection of 3.3% was recorded in trading.

Primary school level has the highest prevalence of 83.6% of *T. gondii* followed by 77.8% in none and the lowest value of 58.7% was recorded in Tertiary while the highest prevalence of HIV infection of 2.9% and co-infection of 1.8% was recorded in secondary.

The highest prevalence of 100.0% of *T. gondii* was recorded in Alajue followed by 84.9% in Ifewara while the lowest value of 57.8% was recorded in Ife. There is significance difference in the prevalence of *T. gondii* in the communities (P= 0.000). The highest prevalence of HIV infection of 7.8% **(**p=0.016**)** and co -infection of 4.4% was recorded in Ile -Ife whiles the lowest prevalence of 1.4% was recorded in Ikire and 1.2% in Ifewara.

The highest prevalence of *T. gondii* infection of 100.0% was recorded in women with blood group AB negative followed by blood group A positive 80.4%, while the lowest prevalence of 30.0% was

recorded in women with blood group A negative and the highest prevalence of HIV infection of 6.3% was recorded in women with blood group B negative and O negative each. Co- infection has highest prevalence of 6.3% in blood group B negative and the lowest of 2.1 % in blood group O negative (Table 2).

Table 2 Prevalence of Toxo*plasma gondii*, *Human Immunodeficiency Virus* and their co-infections according to Blood Groups among women of child bearing age in selected communities in Osun State

Blood group	No examined	No infected (%) HIV	No infected (%) *T. gondii*	No infected (%) HIV *and* TOXO
A pos	56	1 (1.8)	45 (80.4)	0 (0.0)
B pos	70	0 (0.0)	51 (72.9)	0 (0.0)
O pos	192	6 (3.1)	147 (76.6)	4 (2.1)
AB pos	14	0 (0.0)	11 (78.6)	0 (0.0)
A neg	10	0 (0.0)	3 (30.0)	0 (0.0)
B neg	16	1 (6.3)	10 (62.5)	1 (6.3)
O neg	32	2 (6.3)	22 (68.8)	1 (3.1)
AB neg	1	0 (0.0)	1 (100.0)	0 (0.0)
Total	391	10 (2.6)	290 (100.0)	6 (1.5)

HIV (X^2) = 5.501, p= 0.599,TOXO (X^2) = 5.840, P= 0.016**,** HIVTOXO (X^2)= 5.626, p= 0.584

A pos,A Rhesus D positive; B pos, B Rhesus D Positive; O pos, O Rhesus D positive; AB pos, AB Rhesus D positive; A neg, A Rhesus D negative; B neg, B Rhesus D negative; O neg, O Rhesus D negative; AB neg, AB Rhesus D negative; TOXO, Toxo*plasma gondii;* HIV, *Human Immunodeficiency Virus*

The highest prevalence of 77.1% of *T. gondii* was recorded in women with one miscarriage followed by 75.0% with three miscarriages while the lowest value of 73.3% was recorded in women with two miscarriages and the highest prevalence of HIV infection of 5.7% was recorded in women with one miscarriage while the lowest prevalence of 2.5% was recorded in women with no miscarriage. Co-infection was highest 2.9% in women with one miscarriage and lowest (1.6%) in women with no miscarriage (Table 3).

Table 3 Prevalence of Toxo*plasma gondii*, *Human Immunodeficiency Virus* and their co-infections according to miscarriages and pregnancy among women of child bearing age in selected communities in Osun State

	No examined	No infected (%) (HIV)	No infected (%) (*T. gondii*)	No infected (%) HIV and TOXO	P value
Miscarriage					HIV (X^2)= 2.450, p=0.654
0	316	8 (2.5)	235 (74.4)	5 (1.6)	HIVTOXO(X^2)= 1.033, p=0.905
1	35	2 (5.7)	27 (77.1)	1 (2.9)	TOXO (X^2)= 5.924 P=0.205
2	30	0 (0.0)	22(73.3)	0 (0.0)	
3	8	0 (0.0)	6 (75.0)	0 (0.0)	
4	2	0 (0.0)	0 (0.0)	0 (0.0)	
Pregnancy					HIV (X^2)= 0.381, p=0.537
Yes	14	0 (0.0)	14 (100.0)	0(0.0)	HIVTOXO(X^2)= 1.226, p=0.634
No	337	10 (2.7)	46 (13.6)	6 (1.6)	TOXO (X^2)= 0.740 P=0.390
Total	291	10 (2.6)	290 (74.2)	6 (1.5)	

TOXO, Toxo*plasma gondii;* HIV, *Human Immunodeficiency Virus*

The highest prevalence of 100.0% of *T. gondii* and 2.7% of HIV infection was recorded in women with no pregnancy while the co-infection 1.6% was also recorded in women without pregnancy.

Discussion and conclusion

In this study the overall prevalence of *Toxoplasma gondii* infection was 74.2%, HIV was 2.6% and co-infection of both was 1.5% among women of childbearing age, the high prevalence of *T. gondii* value recorded in the study is an indication that women in the study are frequently exposed to the parasite. This value was significantly higher that the prevalence of 44.0% reported in women of child bearing age in Benue State, Nigeria.[7] And also higher than those reported from healthy-individual from different part of Nigeria which ranged from 20.83% - 41.13%.[8–10]

The overall prevalence of 2.6% HIV recorded was lesser than that of 8.4% reported by Eaton in 2014, 67% recorded in Ethiopia[11] but higher than that of 0.36% in Benin city, 0.28% in Bendel State[12] and 1.58% in Lesotho.[13] The co-infection of 1.5% in the study is lesser than that of 25.1% in Asia, 49.1% in Latin and Caribbean and 60.7% in North America and Middle East.[4]

The highest prevalence 94.4% of *T. gondii* was recorded in women age range ≥ 40 years while the lowest 63.2% was recorded in women aged 15-19years.This is in agreement with the finding of Mihu *et al*.[14] where an increase in prevalence from 32% in women aged 15-19 years to a peak of 62% in women aged 40-45 years was reported. It is also similar to the report of Sarman *et al.*[15] who reported an increase in the prevalence from 18.1% in women aged 18–25 years to a peak of 40.5% in women aged 40 years and above. The prevalence of HIV of 4.8% recorded among aged 20-24years was more higher than that of 4.3% recorded among aged 35-39 years in the study which is not in agreement with Eaton *et al.,* 2014 where the HIV infection among women of child-bearing age decreases among women aged 15-24

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years and increases among women aged 35-49 years. Additionally, HIV testing was observed by Muyunda et al.[16] to be higher among the older women 25-34 years compared to the young women 15-19 years. In Avert 2019, Young women (aged 15-24years), and adolescent girls (aged 10-19years) in particular, account for a disproportionate number of new HIV infections.[17]

In this study, the prevalence of *T. gondii* infection is significantly (p= 0.0016) higher in women residing in rural areas (78.5%) than in women in urban areas (67.5%). Similar finding was reported by Olariu et al.[18] where high prevalence of *T. gondii* antibodies was recorded in women of child bearing age in rural areas (70%) than in urban areas (48.1%). However, the finding in this study was in contrast with Mwambe et al.[19] who reported higher prevalence in women living in urban areas (41.5%) than those in rural areas (22.0%). The high prevalence of the infection in rural area might be as a result of frequent contact of the women with animals, living in mud-made houses, poor sanitation system and most importantly with low level of education. The highest prevalence of HIV of 4.5% (p= 0.045) and co-infection of 3.2% (p= 0.025) was recorded in peri-urban area in the study this agrees with the work of (Slutsker *et al.,*1994) where women enrolled at town sites were significantly more likely to be HIV infected than village women (11.3 versus 3.9%; *P*<0.001).[20]

Traditional religion has the highest prevalence of 100.0% of *T. gondii,* followed by Islam 85.9% and there was significance difference in the prevalence of *T. gondii* infection and religion (p=0.001) which could be as a result of the consumption and contact with the reservoirs for *T. gondii* tissue cyst and other meats such as goat, chicken, while the highest prevalence of HIV infection of 2.7% recorded in Christianity and co-infection of 2.3% was recorded in Islam.

Within the occupation, the highest prevalence of 84.0% of *T. gondii* was recorded in house wives while the lowest value of 59.3% was recorded in civil servant and the highest prevalence of *Hepatitis* HIV infection of 3.3% and co-infection of 3.3% was recorded in trading. The result in *T. gondii* infection suggests substantial eating of unwashed raw fruits, vegetables and contact with materials contaminated with blood of the reservoirs especially among house wives. The prevalence of *Hepatitis* HIV and Co-infection of HIV and *T. gondii* could be as a result of use of contaminated syringes and needles, unprotected sex, contact with infected blood etc.[21]

The prevalence of *T. gondii* infection (83.6%) was significantly higher in women with primary school education (p= 0.037) and also, HIV (2.9%) was recorded in secondary and co-infection (1.8%) each was higher in women with primary and secondary school education. Educational attainment was strongly associated with HIV testing among women of child bearing age.[16]

The highest prevalence of *T. gondii* infection of 100.0% was recorded in women with blood group AB negative, and the highest prevalence of HIV infection of 6.3% was recorded in women with blood group B negative and O negative each. Co- infection has highest prevalence of 6.3% in blood group B negative.

The highest prevalence of 77.1% of *T. gondii* was recorded in women with one miscarriage and the highest prevalence of HIV infection of 5.7% was recorded in women with one miscarriage. Co-infection was highest 2.9% in women with one miscarriage. The highest prevalence of 100.0% of *T. gondii* and 2.7% of HIV infection was recorded in women with no pregnancy while the co-infection 1.6% was also recorded in women without pregnancy.

The present study provides new epidemiological data on the prevalence of *T. gondii*, HIV and their co-infections in women of childbearing age from selected communities in Osun State, Nigeria. This result shows a high prevalence of *T. gondii* infection (74.2%), HIV (2.6%) and co-infection (1.5%) in the studied population. The findings in this study may serve as useful information for health policy makers for counseling and education programmes on Toxoplasmosis and Human Immunodeficiency Virus during antenatal clinics, and for the implementation at the national level of a screening and prevention programme for pregnant women.

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Conflicts of interests

The authors declared that there are no conflicts of interest.

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